PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PJF01603WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/03803	International filing date *(day/month/year)* 02.09.2003	Priority date *(day/month/year)* 02.09.2002
International Patent Classification (IPC) or both national classification and IPC G01N21/55		
Applicant MEDICAL BIOSYSTEME LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.03.2004	27.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Consalvo, D Telephone No. +49 89 2399-7093

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03803

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-9 as published

Claims, Numbers

1-30 as published

Drawings, Sheets

1/12-12/12 as published

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-14,16-22,24-30
	No:	Claims	1,15,23
Inventive step (IS)	Yes:	Claims	7,9,26,28
	No:	Claims	1-6,8,10-25,27,29,30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item I

Basis of the report

With the communication dated 9 September 2004 no amendments have been filed. Consequently, the objections raised in the written opinion of 26 August 2004 are kept.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited documents

Reference is made to the following documents:

D1: EP-A-1 085 315 (SUISSE ELECTRONIQUE MICROTECH) 21 March 2001 (2001-03-21)
D2: US 2002/051979 A1 (CHEN SHIPING ET AL) 2 May 2002 (2002-05-02)
D3: EP-A-0 455 067 (HOFFMANN LA ROCHE) 6 November 1991 (1991-11-06)
D4: EP-A-0 286 195 (TNO) 12 October 1988 (1988-10-12)
D5: EP-A-0 617 273 (HOFFMANN LA ROCHE) 28 September 1994 (1994-09-28).

2. Novelty and inventive step

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is not new in the sense of Article 33(2) PCT, for the following reasons:

2.1 Independent claim 1

Document D1 discloses, see paragraphs 10-20, a system for detecting a physical, chemical or biochemical reaction comprising:

i) a coherent radiation source for producing an incident wave (31);
ii) a carrier surface (F) for supporting a specimen to be analysed, the carrier surface mounted on a substrate (S) and capable of supporting surface electromagnetic waves;

In document D1, see Figure 6, the discontinuity in the carrier surface is suitable for splitting an incident wave into a surface electromagnetic wave and a first scattered wave. A second discontinuity in the carrier surface of D1 is suitable for generating a second scattered wave from the surface electromagnetic wave.

Thus, the system of D1 is comprising:

iii) means for splitting an incident wave into a surface electromagnetic wave and a first scattered wave; and

iv) means for generating a second scattered wave from the surface electromagnetic wave.

D1 does disclose the use of a detector and, in paragraph 30 of D1, it is mentioned that the detector according to the invention can be one- or two-dimensional. Since a detector suitable for monitoring the interference between the first scattered wave and the second scattered wave can be any detector which is pixelated (so that it can generate two dimensional images for subsequent recording and display) the additional feature of

v) a detector for monitoring the interference between the first scattered wave and the second scattered wave

is also disclosed in D1.

Moreover, although the document D1 is primarily concerned with the excitation of fluorescence and extraction of the emitted output light, attention is drawn to paragraphs 11-13 of D1.
Here it is mentioned that: "The interaction of said substance with said first electromagnetic radiation preferably comprises luminescence, scattering, absorption, chemiluminescence and/or electrochemi-luminescence. Said first and second set of degrees of freedom preferably comprises the diffraction order, the polarization, the guided-mode order, the grating vector and/or the planes of incidence and excidence"

Thus D1 mentions that polarised radiation can be used as incident wave and that the interaction of the substance with the electromagnetic radiation includes scattering.

2.2 The same consideration apply *mutatis mutandis* to the subject-matter of correspondent method claim 23. The subject-matter of claim 15, "A carrier chip.. wherein the conductive film comprises first means for splitting an incident wave.. and

second means for generating a second wave... " is disclosed in D1, see for instance Figure 6, where a structure having discontinuities in the conductive film is showed.

3. Dependent claims 2-6, 8, 11-14, 16-22 and 24, 25, 27, and 29-30

Dependent claims 2-6, 8, 11-14, 16-22 and 24, 25, 27, and 29-30 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to novelty and/or inventive step, the reasons being that all additional features are disclosed or anticipated in documents D1-D5.

4. Dependent Claims 7, 9, 26, and 28

The subject-matter of claims 7, 9, 26, and 28 does not appear to be evident from the cited prior art and could form the basis of an allowable claim.

5. Further deficiencies in the international application

5.1 With respect to the use of the wording "system for", "means for", "capable of" etc. in claim 1 attention is drawn to the Guidelines PCT/GL/ISPE/1 5.23.

5.2 With respect to the use of parenthesis in claims see the Guidelines PCT/IGL/ISPE/1 5.11.

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(75) Inventors/Applicants *(for US only)*: **DENSHAM, Daniel, Henry** [GB/GB]; The Old Mill, Beaston Cross, Broadhempston, Totnes, Devon TQ9 6BX (GB). **ALIEVA, Elena** [RU/GB]; Mobious Genomics Ltd., Innovation Centre, University of Exeter, Rennes Drive, Exeter EX4 4RN (GB). **GUREVICH, Leonid** [RU/GB]; Mobious Genomics Ltd., Innovation Centre, University of Exeter, Rennes Drive, Exeter, EX4 4RN (GB). **JERDEV, Artem** [RU/GB]; Mobious Genomics Ltd., Innovation Centre, University of Exeter, Rennes Drive, Exeter, EX4 4RN (GB). **KONOPSKY, Valery** [RU/GB]; Mobious Genomics Ltd., Innovation Centre. University of Exeter, Rennes Drive, Exeter, EX4 4RN (GB).

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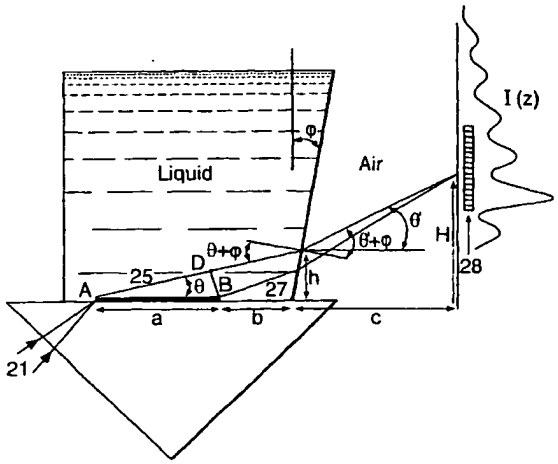
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: BIOSENSOR



(57) Abstract: The present invention relates to a system and method for detecting a physical, chemical or biochemical reaction. The system of the present invention comprises a coherent radiation source for producing an incident wave (21); a carrier surface (AB) for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW); means for splitting the incident wave into an SEW and a first scattered wave (25), wherein the SEW propagates along the carrier surface and interacts with the specimen; means for generating a second scattered wave (27) from the SEW; and, a detector (28) for monitoring the interference between the first scattered wave and the second scattered wave. The invention also relates to carrier surfaces for use on the system. A carrier chip according to the present invention comprises a dielectric substrate; and a conductive film formed on the surface of the substrate suitable for carrying the specimen; wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/GB 03/03803

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/55 G01N21/77

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 085 315 A (SUISSE ELECTRONIQUE MICROTECH) 21 March 2001 (2001-03-21) paragraphs '0010!-'0020!; figures 1-4,17 ---	1-30
X	US 2002/051979 A1 (CHEN SHIPING ET AL) 2 May 2002 (2002-05-02) paragraphs '0172!-'0175!; figures 10-12 ---	1-30
X	EP 0 455 067 A (HOFFMANN LA ROCHE) 6 November 1991 (1991-11-06) column 7, line 41 -column 11, line 43; figures 3A-10 ---	1-30
A	EP 0 286 195 A (TNO) 12 October 1988 (1988-10-12) column 5, line 57 -column 7, line 40; figures 5-8C --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2003	02/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Consalvo, D

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01 69209 A (INST POLYMERUTVECKLING AB ;JOHANSEN KNUT (SE)) 20 September 2001 (2001-09-20) page 7, line 1 -page 8, line 25; figures 1A-4	1-30
A	LENFERINK A T M ET AL: "AN IMPROVED OPTICAL METHOD FOR SURFACE PLASMON RESONANCE EXPERIMENTS" SENSORS AND ACTUATORS B, ELSEVIER SEQUOIA S.A., LAUSANNE, CH, vol. B3, no. 4, 1 April 1991 (1991-04-01), pages 261-265, XP000243208 ISSN: 0925-4005 abstract; figure 1	1-30
X	EP 0 617 273 A (HOFFMANN LA ROCHE) 28 September 1994 (1994-09-28) column 3, line 33 -column 5, line 50	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB 03/03803

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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525, 589

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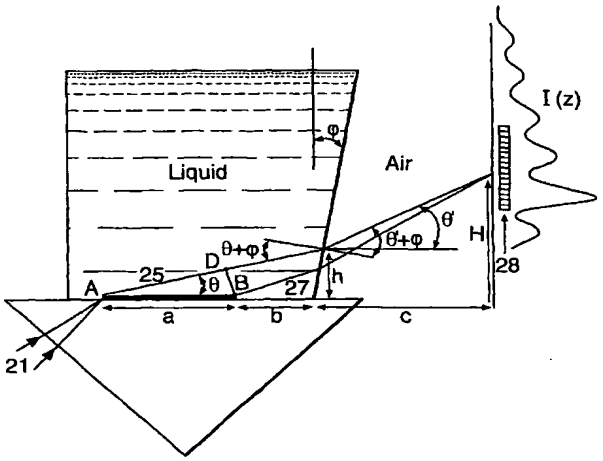
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: BIOSENSOR



(57) Abstract: The present invention relates to a system and method for detecting a physical, chemical or biochemical reaction. The system of the present invention comprises a coherent radiation source for producing an incident wave (21); a carrier surface (AB) for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW); means for splitting the incident wave into an SEW and a first scattered wave (25), wherein the SEW propagates along the carrier surface and interacts with the specimen; means for generating a second scattered wave (27) from the SEW; and, a detector (28) for monitoring the interference between the first scattered wave and the second scattered wave. The invention also relates to carrier surfaces for use on the system. A carrier chip according to the present invention comprises a dielectric substrate; and a conductive film formed on the surface of the substrate suitable for carrying the specimen; wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BIOSENSOR

Field of the Invention

The present invention relates to a system for detecting a physical, chemical or biochemical reactions, and in particular to a system in which surface electromagnetic waves (SEWs) interact with a specimen involved in the reaction.

Background to the Invention

Biosensors incorporating surface electromagnetic wave technology (and, in particular, surface plasmon resonance - SPR - sensors) are increasingly gaining popularity in pharmaceutical, medical and environmental applications as well as in biochemical research. These type of sensors require no labelling and offer the possibility of real-time monitoring of binding events. They are based on the sensitivity of surface electromagnetic waves (SEW) to the refractive index of the thin layer adjacent to the surface where the SEW propagates. In a typical biosensor application, one binding partner is immobilized on the surface (often called a target) and the other partner is flowed across it. As binding occurs, the accumulation or redistribution of mass on the surface changes the local refractive index that can be monitored in real time by the sensor.

Several methods of SPR registration have been proposed and realized in biosensors. The most popular methods are based on Kretschmann-Raether configuration where intensity of the light reflected from sensor is monitored. This technique, considered to be one of the most sensitive, is described in J. Homola et al, Sensors and Actuators B 54, p.3-15 (1999) and has a detection limit of $5x10^{-7}$ refractive index units. Measuring SPR phase changes can further increase the sensitivity of the sensor by one or two orders of magnitude. This is described in Nelson et al, Sensors and Actuators B 35-36, p.187 (1996) and in Kabashkin et al, Optics Communications 150, p.5 (1998). Prior art interferometric devices such as a Mach Zehnder device have been configured to measure variations in the refractive index at the sensor surface via phase shifts. This is disclosed in WO01/20295. The configuration requires four independent components and is sensitive to sub-wavelength relative displacements of these components and hence very small mechanical and environmental perturbations. A mechanically more robust monolithic interferometric design is outlined in WO03014715.

However, although the theoretical limit for the sensitivity can be as good as 10^{-8} refractive index units, the sensitivity of real systems is limited to 10^{-6} due to fluctuations in the temperature and chemical composition of the buffer surrounding the sample. For example, to achieve a sensitivity of 10^{-7} refractive index units a temperature stability better than 10^{-3} °C would be required. This is due to the fact that the influence of changes in the refractive index of the surrounding buffer cannot be isolated from the influence of changes in thickness and refractive index of the analyte absorbed on sensor surface using the methods and systems of the prior art.

Summary of the Invention

An object of the present invention is to provide a surface electromagnetic wave (SEW) sensor system that can compensate for changes in the bulk refractive index of a buffer or allows the contribution of the bulk refractive index to an interference pattern to be separated from the contribution of an analyte absorbed on the sensor surface. The invention relates to a sensor system, a sensor method and carrier chip designs for use in a sensor.

According to a first aspect of the present invention a system for detecting a physical, chemical or biochemical reaction comprises:

a coherent radiation source for producing an incident wave;

a carrier surface for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW);

means for splitting the incident wave into an SEW and a first scattered wave, wherein the SEW propagates along the carrier surface and interacts with the specimen;

means for generating a second scattered wave from the SEW; and,

a detector for monitoring the interference between the first scattered wave and the second scattered wave.

According to a second aspect of the present invention a carrier chip for a specimen to be monitored, comprises:

a dielectric substrate; and

a conductive film formed on the surface of the substrate suitable for carrying the specimen;

wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the

SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

According to a third aspect of the present invention a method of monitoring a specimen undergoing a physical, chemical or biochemical reaction occurring on a surface supporting surface electromagnetic waves (SEW), comprises the steps of:

splitting an incident wave into a first scattered wave and SEW such that the SEW propagates along the surface and interacts with the specimen;

splitting the SEW which has interacted with the specimen to generate a second scattered wave; and,

monitoring the interference pattern between the first and second scattered waves.

Brief Description of the Drawings

Examples of the present invention will now be described with reference to the accompanying drawings in which:

Figure 1 is a schematic illustration of an apparatus according to the present invention for detecting a physical, chemical or biochemical reaction;

Figure 2 illustrates a first embodiment of a system according to the present invention in which changes in bulk refractive index are compensated for a particular angle so that the system is only sensitive to the changes in thickness or refractive index of an analyte absorbed on the sensor surface;

Figure 3 illustrates a second embodiment of a system according to the present invention;

Figure 4 shows detail of a carrier chip according to the present invention;

Figure 5 shows another embodiment of a carrier chip according to the present invention;

Figure 6 shows the calculated variation of the interference fringe position at the optimal angle in the embodiment of Figure 2 versus bulk refractive index of a surrounding buffer;

Figure 7 illustrates measurements made using the embodiment of Figure2 where the buffer (water) surrounding the chip was cooled down from 46 °C to 22 °C. The peak position ("phase") is insensitive to the bulk refractive index changes associated with heating while peaks separation ("frequency") is.

Figure 8 illustrates a further carrier chip according to the present invention;

Figure 9 illustrates an embodiment of a multi-point array detection system system according to the present invention in which the whole sensor surface is simultaneously illuminated using a line source and a 2-D CCD-array is used for detection;

Figure 10 shows an example of an image observed on the CCD-array shown in Figure 9;

Figure 11 shows multi-track analysis of streptavidin binding to a carboxymethylated surface along the sensor line. Tracks on the graph correspond to points along the sensor line separated by approximately 40 μm; and,

Figure 12 shows an embodiment of a 2-D sensor array.

Detailed Description

Figure 1 shows schematically a system for monitoring a physical, chemical or biochemical interaction in accordance with a first aspect of the present invention. A coherent optical beam generated by a monochromatic laser is focused using a lens, onto the edge of a metallic film able to support surface electromagnetic waves (SEWs). The optical beam passes through the glass prism on which the metallic film is mounted. A near-infrared laser 11 is used as the illumination source. Using a near-infrared source has the advantage of long propagation length for surface plasmons in gold and silver while conventional optics can be still used for imaging and illumination. However, other monochromatic sources are suitable and may be used.

The laser provides a p-polarised beam. The p-polarised laser beam passes through the focusing lens 12 and then through the glass prism 13 on which a substrate 14 with a microfabricated metal film is attached, using an index matching liquid or gel in a fluidic cell. The index matching gel reduces light scattering and creates a continuous optical path. The glass prism may be a triangular prism as shown or a hemi-cylindrical prism. The laser beam is focused on an edge of the structure 13. The laser beam falls on the glass/liquid interface at an incidence angle larger than the angle of total internal reflection, so that the laser beam is totally reflected except at a small area around the edge of the metal structure. At the edge of the structure the evanescent light wave formed on reflection is partly scattered into light 15 propagating through the fluidic cell and partly scattered into a plasmon wave 16 propagating along the metal structure. The plasmon wave is further scattered by the structure to produce light wave 17. Waves 15 and 17 propagate

through the liquid cell and produce an interference fringe pattern on the measurement device 18.

The metal structure can be formed from gold or silver, or any other metal capable of supporting surface plasmons or a combination of them, or alternatively a dielectric multilayer supporting a SEW. It is preferred to use either gold or silver/gold multilayer to increase surface plasmon propagation length. The metal structure can be deposited on the prism using a lithographic process. The metal structure is described below in more detail with reference to Figures 4 and 5 below.

The method of the measurement is further illustrated by Figure 2. As the phase is conserved during scattering processes the volume radiation beams 25 and 27 can be brought to interference. The phase difference between the beams depends on a surface plasmon (or SEW) wave vector k_{sp}, the distance between the two scattering points *a* and the refractive index of the solution *n*. As the refractive index of the solution *n* and plasmon wave vector k_{sp} change, the shift of the interferogram will be detected by a sensor 28 that can be either 2-section photodiode, 1-D photodetector or CCD array, or 2-D photodetector or CCD array.

The phase difference between beams 25 and 27 can be written as

$$2 \cdot \pi \cdot a \cdot (n_{sp} - n \cdot \cos(\theta)) / \lambda \ ,$$

where λ – is the wavelength of the light and n_{sp} is related to *n* via following equation:

$$n_{sp} = \sqrt{\frac{\varepsilon_m \cdot n_l^2}{\varepsilon_m + n_l^2}} .$$

The bulk refractive index *n* of the buffer fluid and the local refractive index n_l next to the metal surface are distinct and can differ due to layers physically or chemically absorbed on the metal surface (i.e. bound analyte). It can be assumed that:

$$n_l = n + \Delta n .$$

The direction to any particular point on the interference pattern is:

$$\cos(\theta) = \frac{1}{n}(n_{sp} - (m + \Delta m) \cdot \lambda / a) .$$

where *m* is an integer for a maximum, a half-integer for a minimum, or any other number for an arbitrary fixed point on the interference pattern and Δm is an additional phase shift upon excitation and detachment of a SEW. The direction θ of this particular point depends both on *n* and Δn, but expanding the above equation

into a series and differentiating it can be found that changes in bulk refractive index in this geometry are partly compensated and the system is more sensitive to the refractive index changes on the surface by a factor of:

$\frac{\partial\theta / \partial n}{\partial\theta / \partial\Delta n} \approx \frac{n^2}{\varepsilon_m}$ (this factor is of order 10 for gold and silver).

Full immunity to the bulk refractive index variation cannot be achieved as the optical path in the solution $n^*|AD|$ is always smaller than the one along the metal structure n_{sp}^*a, as shown in Figure 2.

Nevertheless, this suppression factor can be further improved or n and Δn separated by varying the shape of the fluidic cell. For example the optical path length of the interfering rays for a particular point m can be equalized as shown in Figure 2. The direction to the detector θ' is connected to θ via Snellius law:

$$n \cdot \sin(\theta + \varphi) = \sin(\theta' + \varphi)$$

where Φ is the tilt angle of the cell's exit wall. This equation can be used to find an angle θ where the variation in n is not reflected in θ' by solving:

$$\frac{\partial}{\partial n}(n \cdot \sin(\theta + \varphi)) = 0.$$

The solution is:

$$\varphi_m = \arctan\left(\frac{(m+\Delta m)\lambda - an_{sp}^3 / \varepsilon_m}{\sqrt{a^2 n^2 - (an_{sp} - (m+\Delta m)\lambda)^2}}\right) - \arccos\left(\frac{an_{sp} - (m+\Delta m)\lambda}{an}\right).$$

At such a tilt angle Φ a small variation of n will not change the direction θ' on the given point m. The calculations illustrating this are shown in Figure 6. As can be seen from Figure 6, significant variation of buffer refractive index (equivalent to heating the water surrounding the sample by ~100°C) produces a negligible shift (within 1μm) in the fringe position at the optimal angle. On the other hand the sensitivity to the variation in Δn will stay the same.

We can also find an optimum number m for a given value of Φ. For example for the right wall (Φ =0):

$$m \approx 2\frac{an^3}{\lambda|\varepsilon_m|} \text{ and } \theta = \arccos\left[\left(\frac{\varepsilon_m + n^2}{\varepsilon_m}\right)^{3/2}\right].$$

For silver film (ε_m =-53) surrounded by water (n=1.326) the optimal angle θ≈18°. The part of the fringe pattern located at the optimal angle will not move if only the bulk refractive index varies and the rest of the pattern will breathe around it. Figure 7

shows the experimentally observed variation of a peak position at the optimal angle while cooling the water surrounding the silver microstructure from 46°C down to 22°C (refractive index variation ~$2*10^{-3}$). The lower line shows that the peak position (phase) is insensitive to bulk refractive index changes, whilst the upper line shows that the peak separation (frequency) varies considerably.

Alternatively, the bulk refractive index can be measured simultaneously and subtracted during data analysis. In the case of a semi-cylindrical cell as illustrated in Figure 3, where the first edge of the structure is aligned to the geometrical center of the cell, the interference fringes on the detector 38 are equidistant (this simplifies harmonic analysis of the pattern) and the distance between interference fringes ("frequency") depends only on the refractive index of the buffer while fringe position depends both on n and Δn.

Figure 4 shows a close up of the profile of a film for use in the apparatus of Figure 1, Figure 2 or Figure 3 in accordance with a second aspect of the present invention. As shown in Figure 4 a carrier film is mounted on the surface of a supporting transparent dielectric material. In this embodiment the carrier film includes a first section 41 of a first thickness and a second section 42 of greater thickness. Coherent radiation 43 incident on the edge of the first section in an attenuated total reflection geometry (ATR), i.e. incident under angle larger than the angle of total internal reflection, will partly scatter into volume radiation 45 and partly generate a SEW 44. This SEW 44 will propagate along section 41 of the carrier film until it reaches the boundary between sections 41 and 42. At this boundary the SEW will again scatter generating a volume radiation 46.
In another embodiment shown in Figure 5, coherent incident radiation 51 is incident on a flat part 52 of a carrier film generating SEW 53 which travels along the section 52 towards the edge of a second section 54. There it is partially scattered into volume radiation 55 and partially transmitted along section 54. The SEW will further travel to the opposite edge of the section 54 where it will be scattered into volume radiation 56.

Further film designs are possible, incorporating the features of both Figures 3 and 4. It is also possible to induce scattering from the surface of the film by introducing a change in the refractive index of the film or surrounding materials at the point at which scattered waves are to be generated. Different means of SEW generation known in the art can be envisaged such as using small apertures or arrays of apertures or using gratings.

It is also envisaged that the SEW could be routed along the carrier surface and/or focused on an area of interest using plasmonic circuitry known in the art. This is described in: F.R. Aussenegg et al., Opto-electronic review 10, p.217 (2002). Plasmonic circuitry to this end could be formed lithographically.

Figure 8 shows another possible embodiment of a microfabricated sensor with a built-in reference area. The incident beam is scattered not only on the metal film 81 (as in Fig. 4) but also on the film 87 (which can be both metallic or non metallic) generating a third volume wave 88. The relative phase of the wave 88 will depend on the bulk refractive index only. If the gap between 81 and 87 is different from the length of 81, the contribution of the wave 88 to the fringe pattern can be separated by harmonic analysis.

The above described system can be readily converted into a multi-point array detection system. A possible embodiment of such system is shown on Figure 9. A laser beam 92 generated by a laser 91 goes through a beam expander and conditioner 93 and is focused into a line by the cylindrical lens 94. Scanning mechanism 95 can switch the laser line between a number of microfabricated structures located on a substrate 96. The interference pattern generated by the microstructures is imaged and projected on a CCD-camera 98 by an optical system 97. In the particular embodiment of the optical system described, the image on the CCD-camera is composed of a set of interference patterns generated by every point along the structure. This is shown in Figure10. As there is one to one correspondence between the interference patterns on CCD and points along the structure, every particular location can be traced during a biochemical experiment. This is shown in Figure 11. The system can be used to monitor binding of different analytes to target areas in DNA or protein arrays. In this case, the substrate can be spotted with different targets, illustrated schematically in Figure 12, where target material 121 is spotted on a plurality of microstructures 122 fabricated, for example, according to embodiments of Figures 4, 5. These microstructures can be interrogated either sequentially or simultaneously.

It is further recognised that if the microstructures have different width, as shown in Figure12, the spatial frequency of the interference patterns they produce will be different and their individual signals can be separated by harmonic analysis.

The above described system is particularly suitable for detecting the generation of the complementary base pairs in a strand of DNA. A complimentary DNA strand can be produced using a polymerase and a parent DNA strand. A DNA

strand is built from four base blocks, and binding of each of these four blocks to a DNA strand will lead to a characteristic charge distribution in a polymerase on the surface of the film. This in turn will lead to a characteristic change in the phase velocity of the SEW and hence a characteristic change in the interference pattern. The use of surface plasmon resonance in the detection of nucleotide incorporation during DNA synthesis, is disclosed in WO-A-99/05315, the content of which is hereby incorporated by reference.

In order to increase accuracy a number of identical specimens can be placed along the length of the film so as to give rise to a greater interaction length between the specimen and the plasmons. Characteristic phase changes for particular reactions can be found by monitoring known reactions under known conditions.

CLAIMS

1. A system for detecting a physical, chemical or biochemical reaction comprising:

a coherent radiation source for producing an incident wave;

a carrier surface for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW);

means for splitting the incident wave into an SEW and a first scattered wave, wherein the SEW propagates along the carrier surface and interacts with the specimen;

means for generating a second scattered wave from the SEW; and,

a detector for monitoring the interference between the first scattered wave and the second scattered wave.

2. A system according to claim 1, wherein the incident wave is a SEW.

3. A system according to claim 1 or 2, wherein the means for splitting the incident wave is a discontinuity in the carrier surface.

4. A system according to any preceding claim, wherein the means for generating the second scattered wave is a discontinuity in the carrier surface.

5. A system according to claim 3 or 4, wherein the discontinuity is a discontinuity in the thickness of the carrier surface.

6. A system according to claim 3 or 4, wherein the discontinuity is a discontinuity in the refractive index of the carrier surface or adjacent materials.

7. A system according to any preceding claim, wherein the specimen is contained in a reaction vessel containing a reaction fluid, and wherein at least one scattered wave propagates through the reaction fluid.

8. A system according to claim 7, wherein the detector is positioned outside the reaction vessel.

9. A system according to claim 8, wherein the reaction vessel is shaped relative to the position of the carrier surface and the position of the detector so as to minimise the effect of fluctuation in the refractive index of the reaction fluid on the interference detected by the detector.

10. A system according to any preceding claim, wherein the SEW is a surface plasmon.

11. A system according to any preceding claim, further comprising a polymerase on the carrier surface suitable for matching complimentary base pairs of a DNA strand, wherein the system is used to monitor a DNA sequencing operation.

12. A system according to any preceding claim, wherein a plurality of areas of the carrier surface can be monitored simultaneously.

13. A system according to any preceding claim, wherein a plurality of areas of the carrier surface can be monitored sequentially.

14. A system according to claim 12 or 13, wherein the carrier surface includes a plurality of structures of different width.

15. A carrier chip for a specimen to be monitored, comprising:

a dielectric substrate; and

a conductive film formed on the surface of the substrate suitable for carrying the specimen;

wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

16. A carrier surface according to claim 15, wherein the first and second means are discontinuities in the conductive film.

17. A carrier surface according to claim 16, wherein the first or second means is a discontinuity in the thickness of the conductor film.

18. A carrier surface according to claim 16, wherein the discontinuity is a discontinuity in the refractive index of the carrier surface or adjacent materials.

19. A carrier surface according to any one of claims 15 to 18, further comprising means for generating a reference scattered wave.

20. A carrier surface according to claim 19, wherein the reference scattered wave is generated from an incident wave.

21. A carrier surface according to claim 19 or 20, wherein the reference scattered wave interferes with both the first or second scatted wave at a different spatial frequency from that at which the first and second scattered waves interfere.

22. A carrier surface according to any one of claims 15 to 21, wherein the second means is an increase in the thickness of the film in the direction of propagation of the SEW.

23. A method of monitoring a specimen undergoing a physical, chemical or biochemical reaction occurring on a surface supporting surface electromagnetic waves (SEW), comprising the steps of:

splitting an incident wave into a first scattered wave and SEW such that the SEW propagates along the surface and interacts with the specimen;

splitting the SEW which has interacted with the specimen to generate a second scattered wave; and,

monitoring the interference pattern between the first and second scattered waves.

24. A method according to claim 23wherein the incident wave is a SEW.

25. A method according to claim 23 or 24, wherein the incident wave is generated by a coherent light source.

26. A method according to any one of claims 23 to 25, wherein the specimen is held within a reaction fluid in a reaction vessel, and at least one of the first and second scattered waves propagates through the reaction fluid.

27. A method according to claim 26, wherein the monitoring of the interference pattern takes place outside of the reaction vessel.

28. A method according to claim 27, wherein the reaction vessel is shaped so as to minimise the effect of fluctuations in the refractive index of the reaction fluid on the interference pattern between the first and second scattered waves.

29. A method according to any one of claims 23 to 28, wherein the specimen includes a polymerase, and the SEW interacts with the polymerase as it incorporates nucleotides into a polynucleotide strand complementary to a target polynucleotide.

30. A carrier surface according to any one of claims 15 to 22, together with an immobilised polymerase enzyme fixed to the carrier surface.

Fig.1.

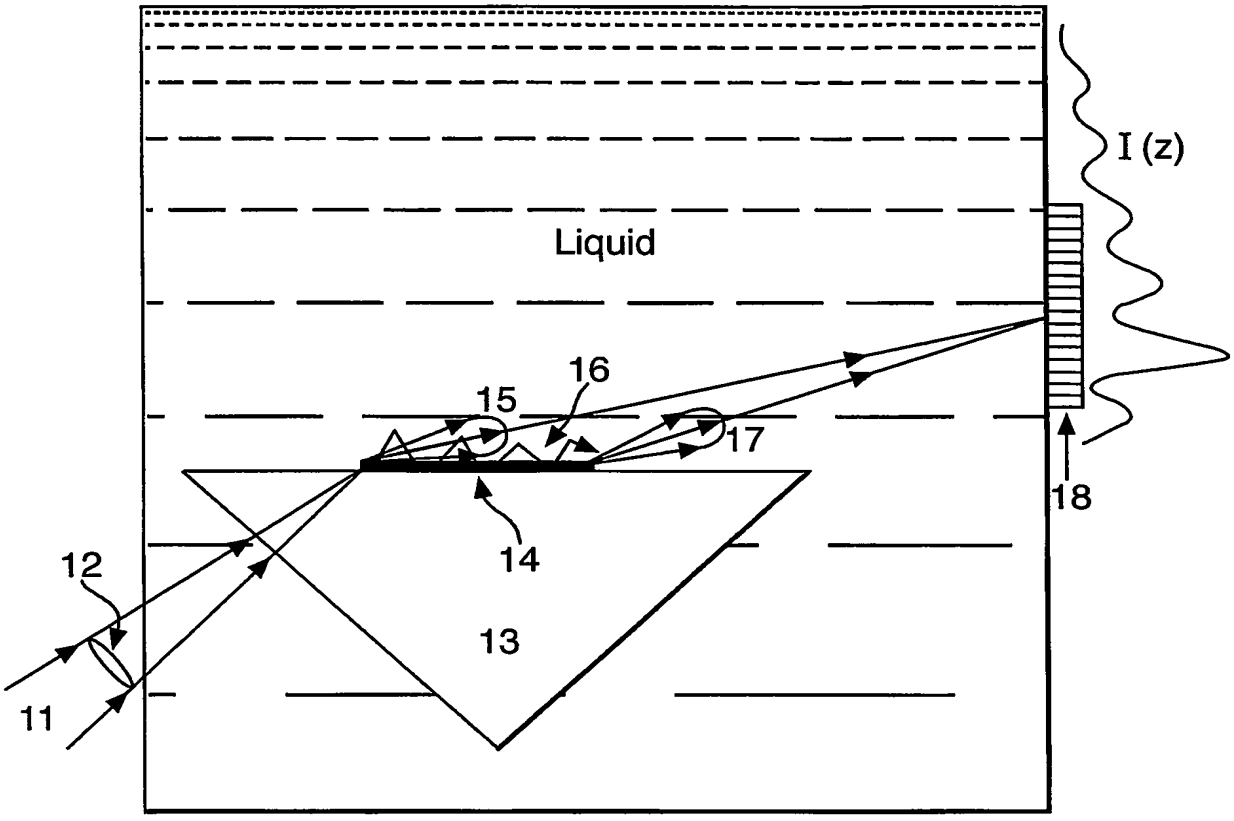


Fig.2.

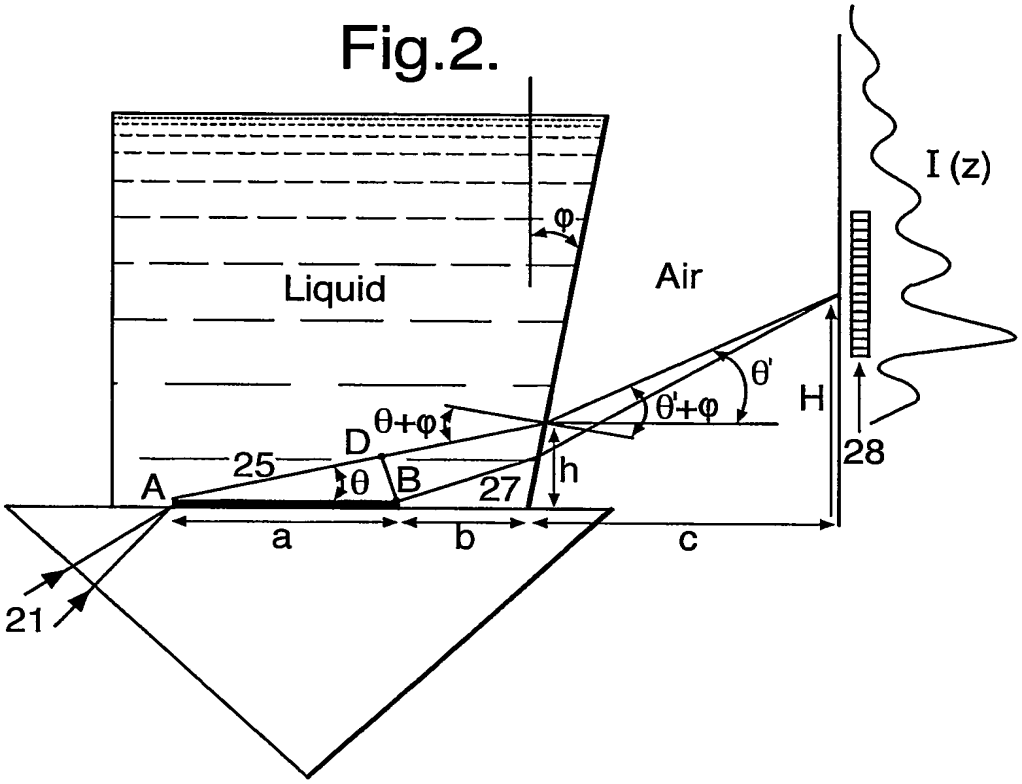


Fig.3.

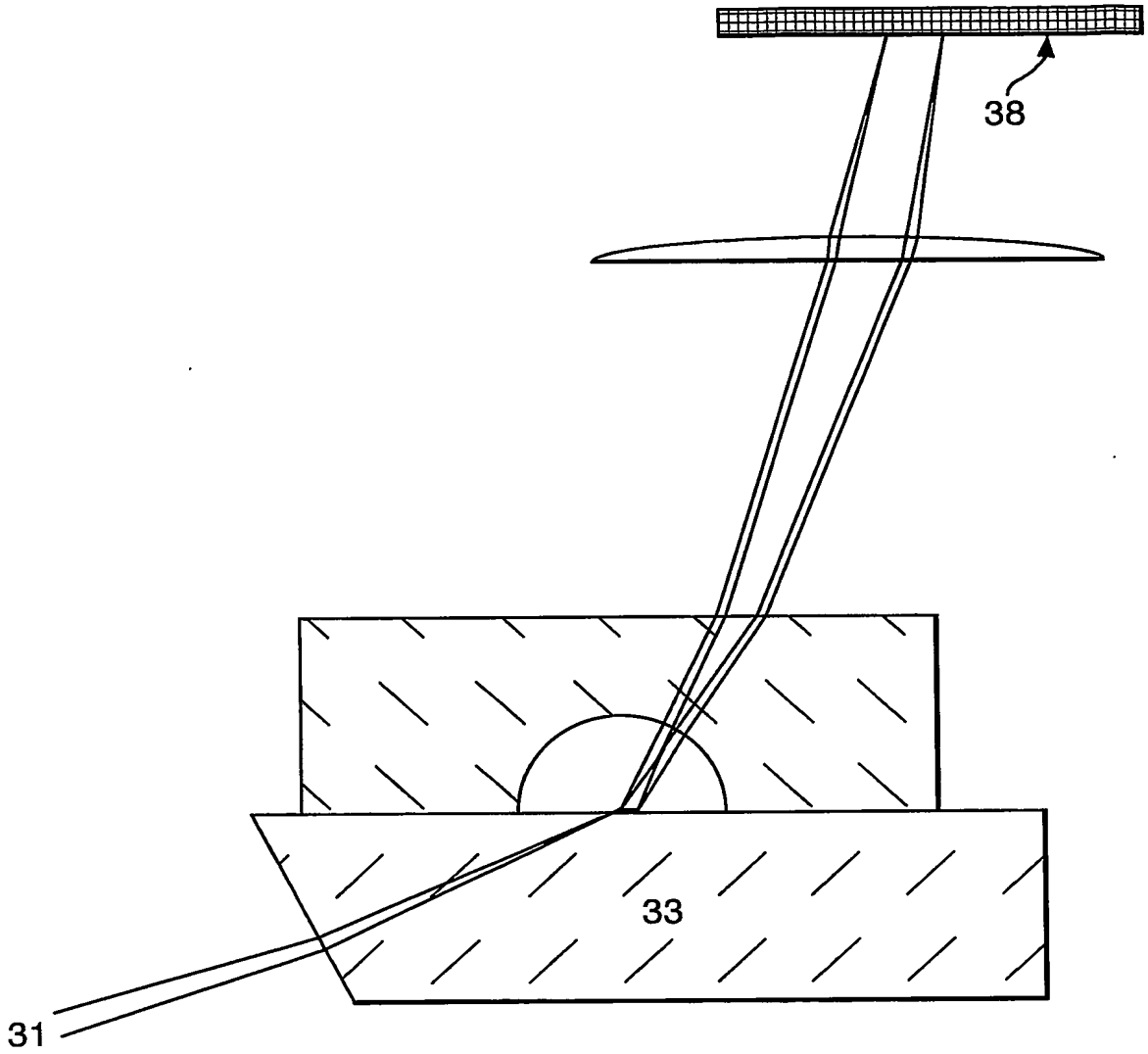


Fig.4.

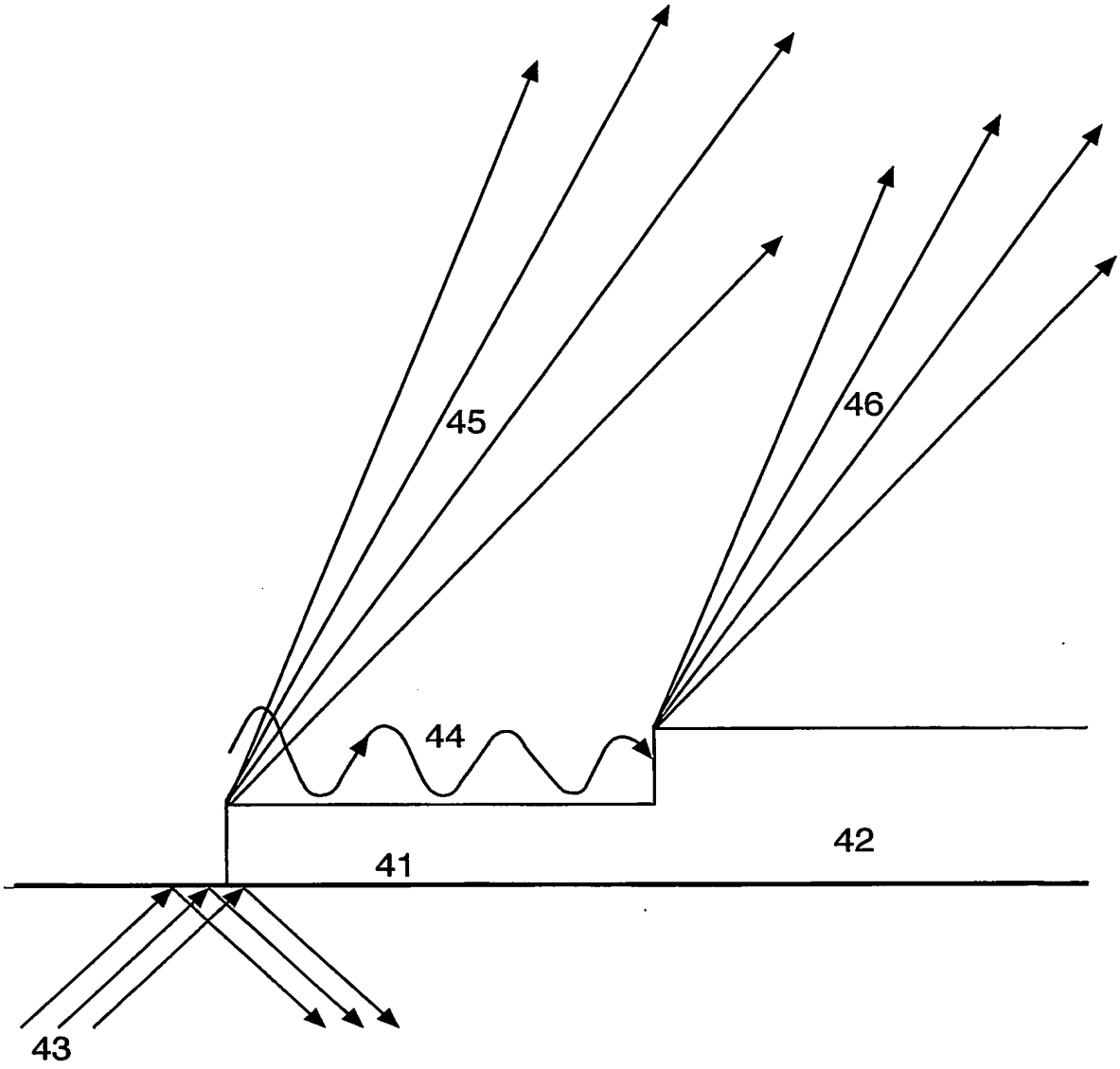


Fig.5.

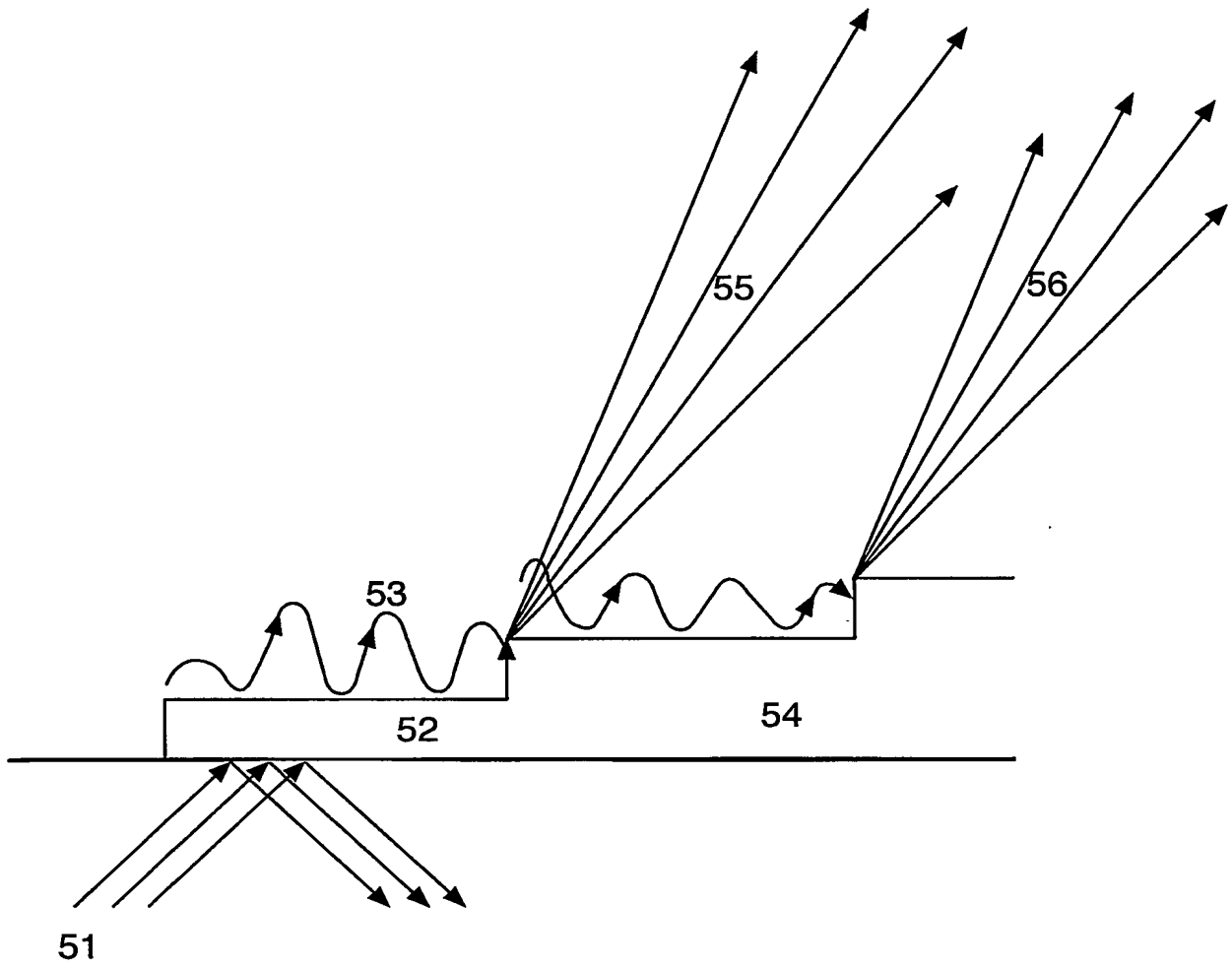
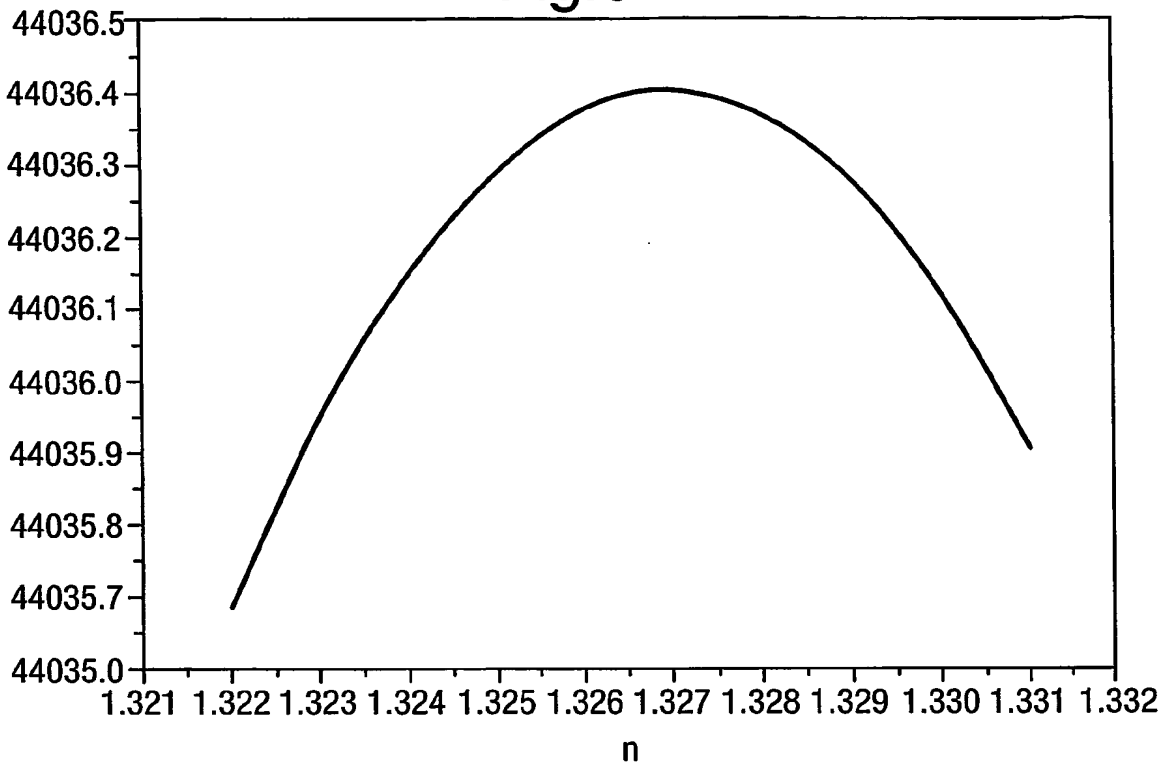


Fig.6.


H, μm
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44036.4
44036.3
44036.2
44036.1
44036.0
44035.9
44035.8
44035.7
44035.0
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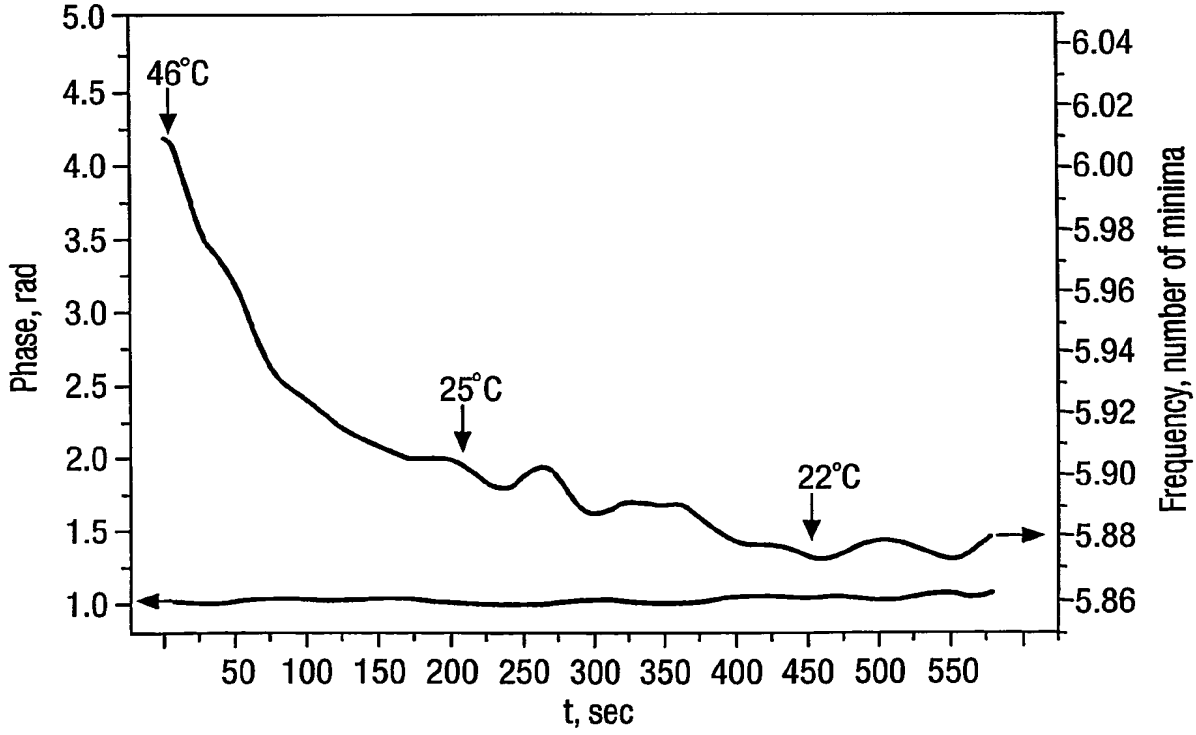
n

Fig.7.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2.0
1.5
1.0
Phase, rad
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25°C
22°C
6.04
6.02
6.00
5.98
5.96
5.94
5.92
5.90
5.88
5.86
Frequency, number of minima
50 100 150 200 250 300 350 400 450 500 550
t, sec

Fig.8.

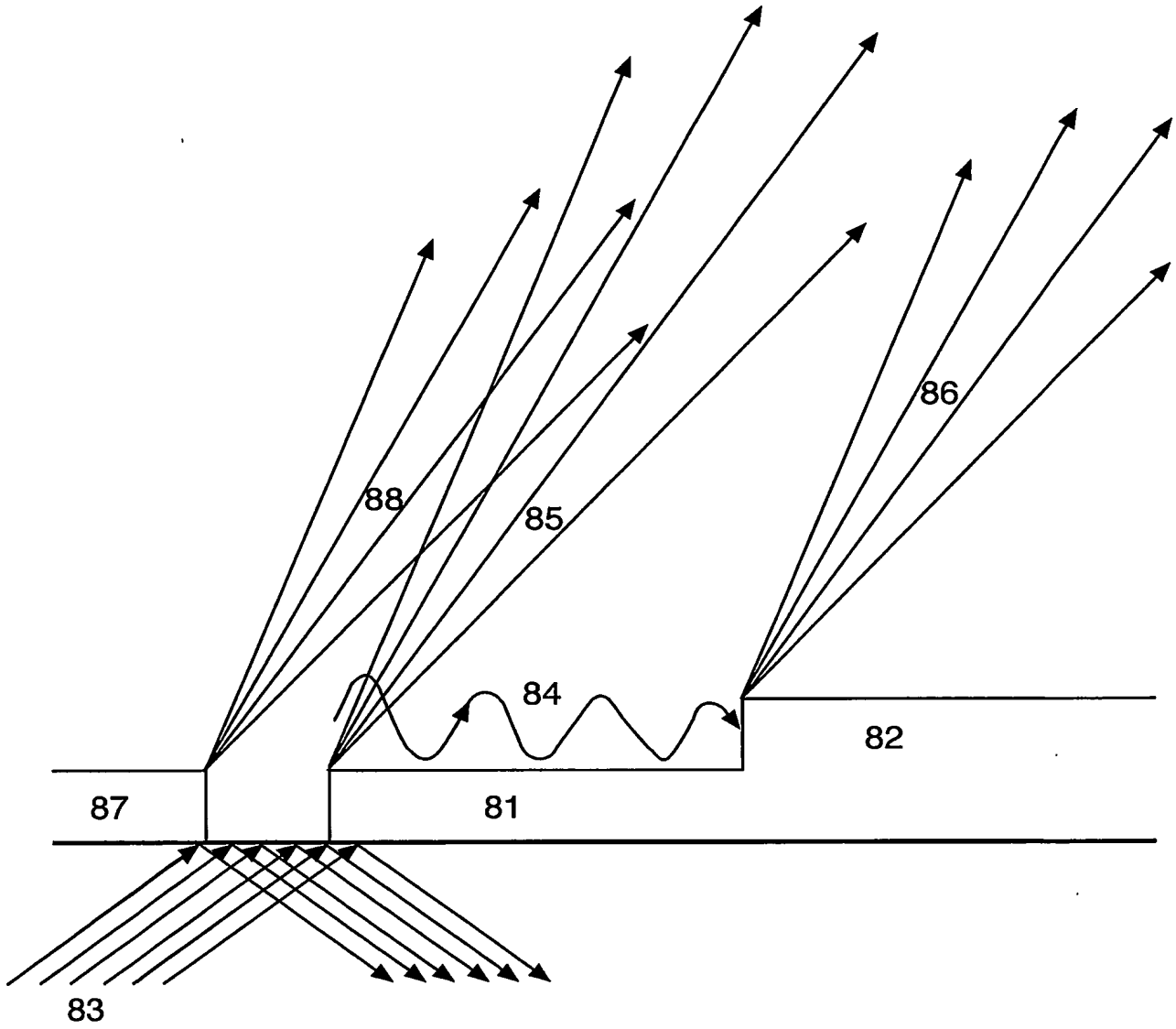


Fig.9.

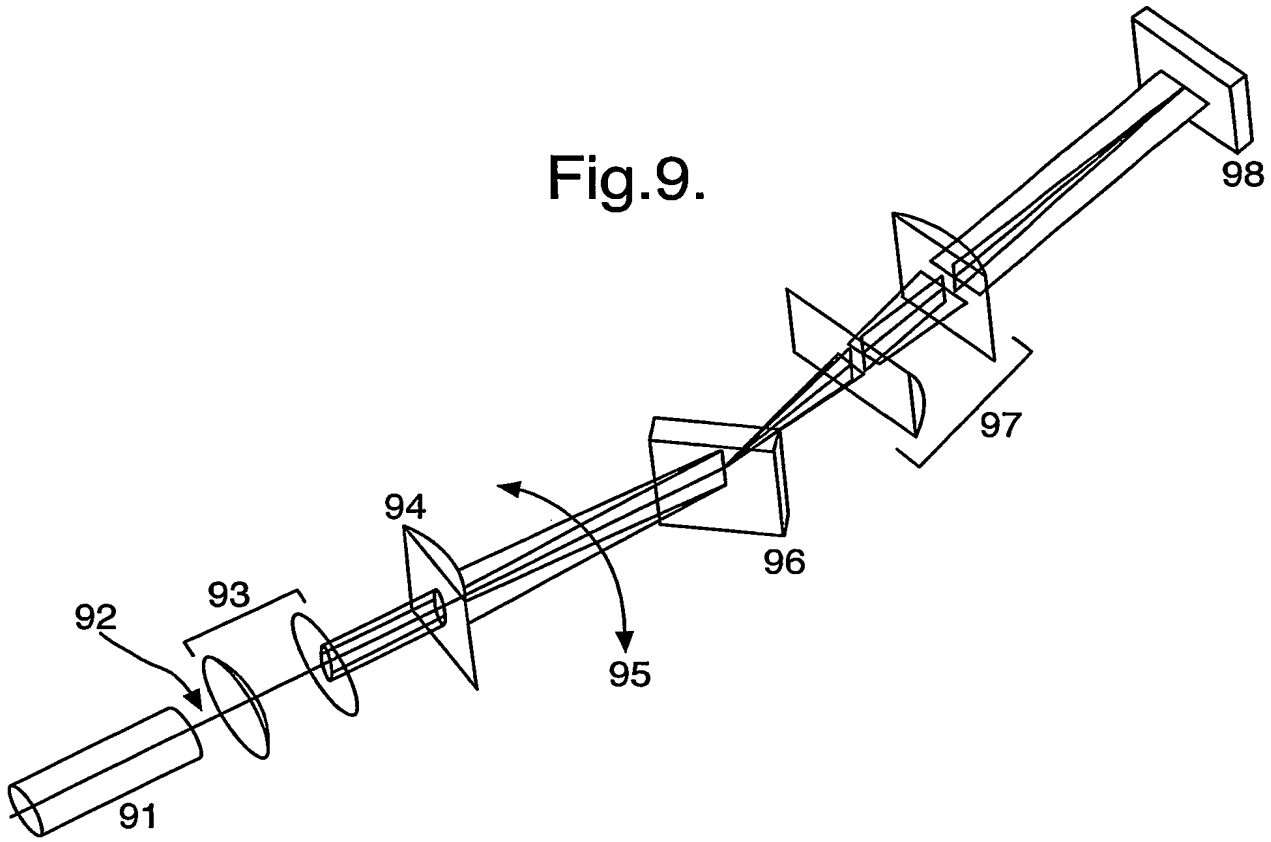


Fig.10.

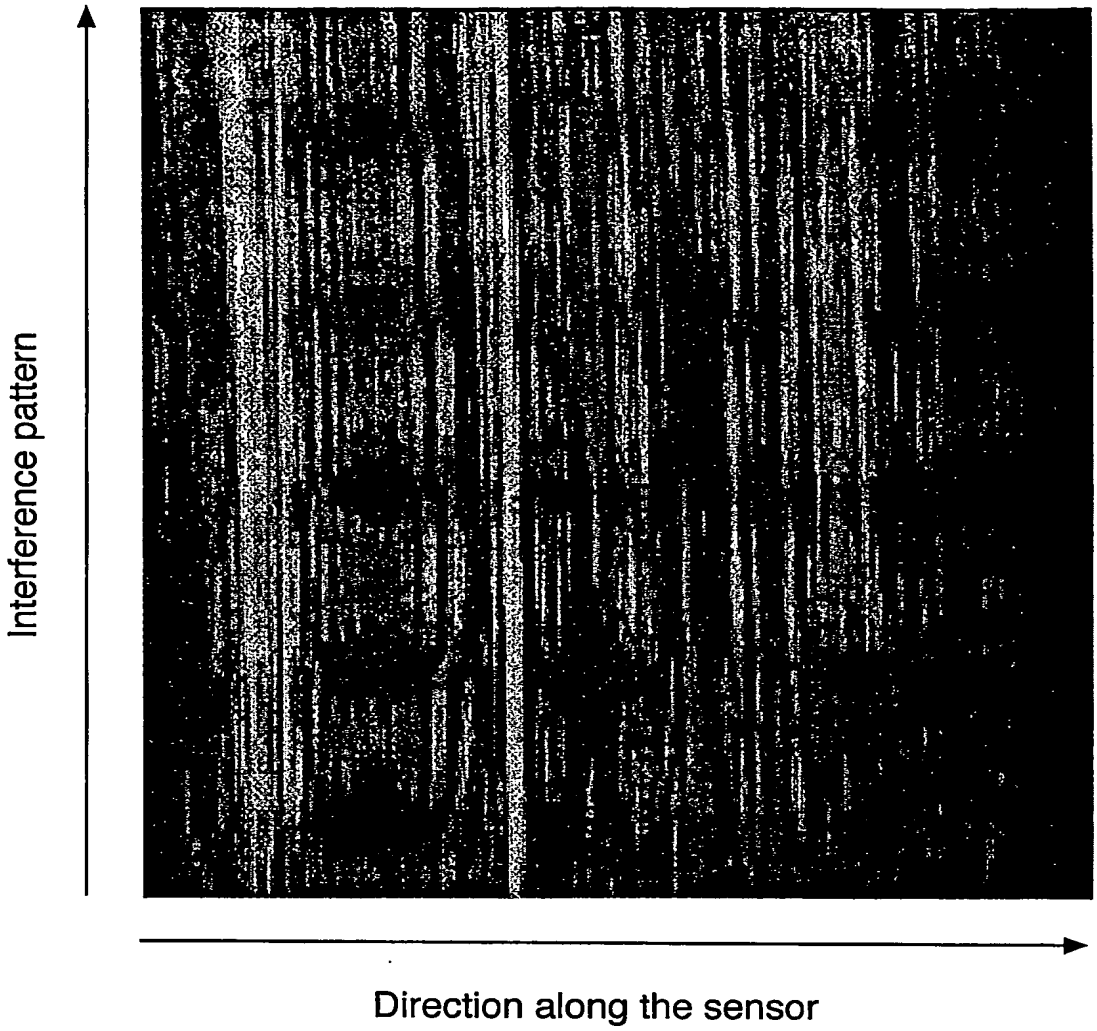


Fig.11.

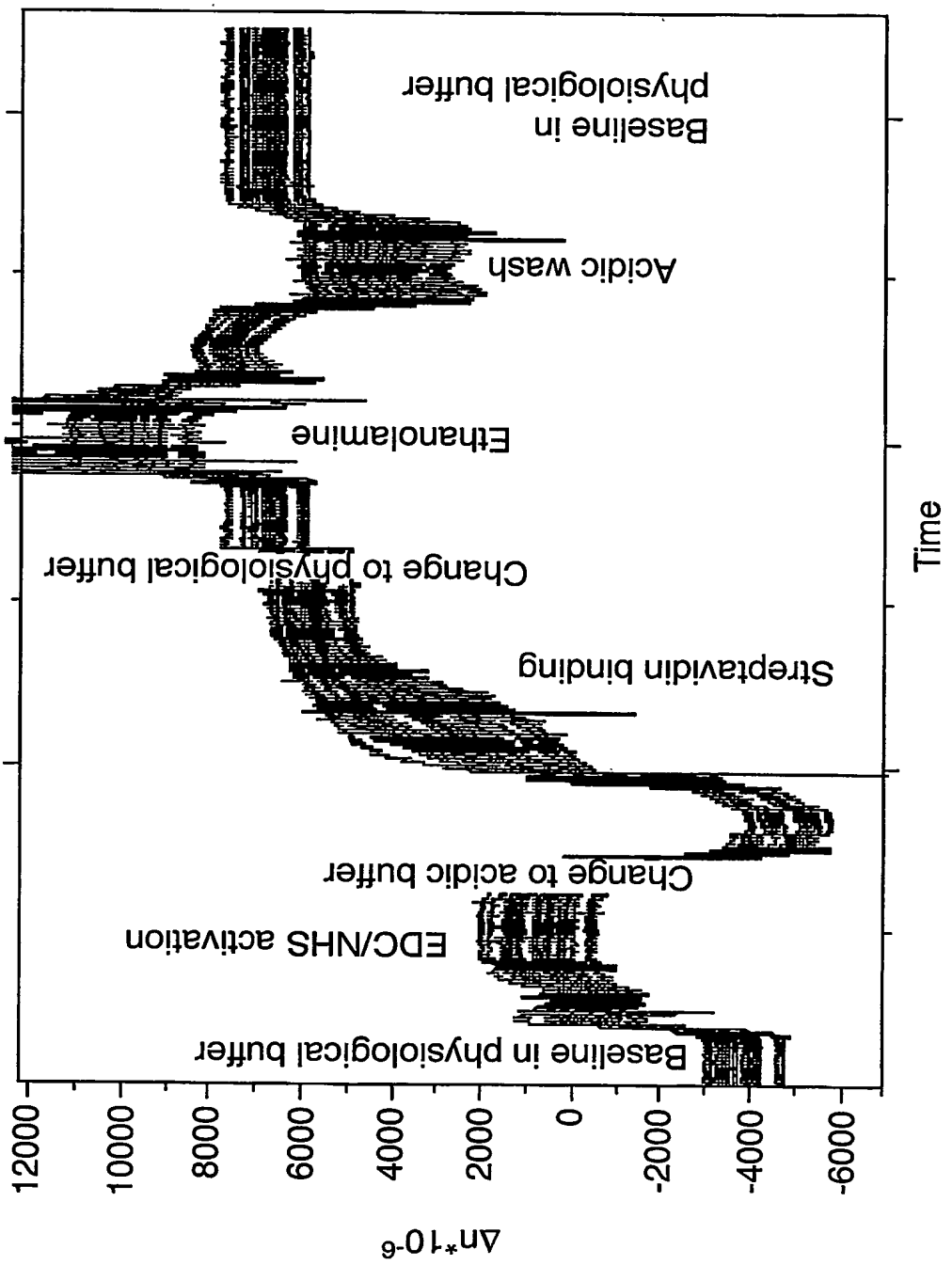
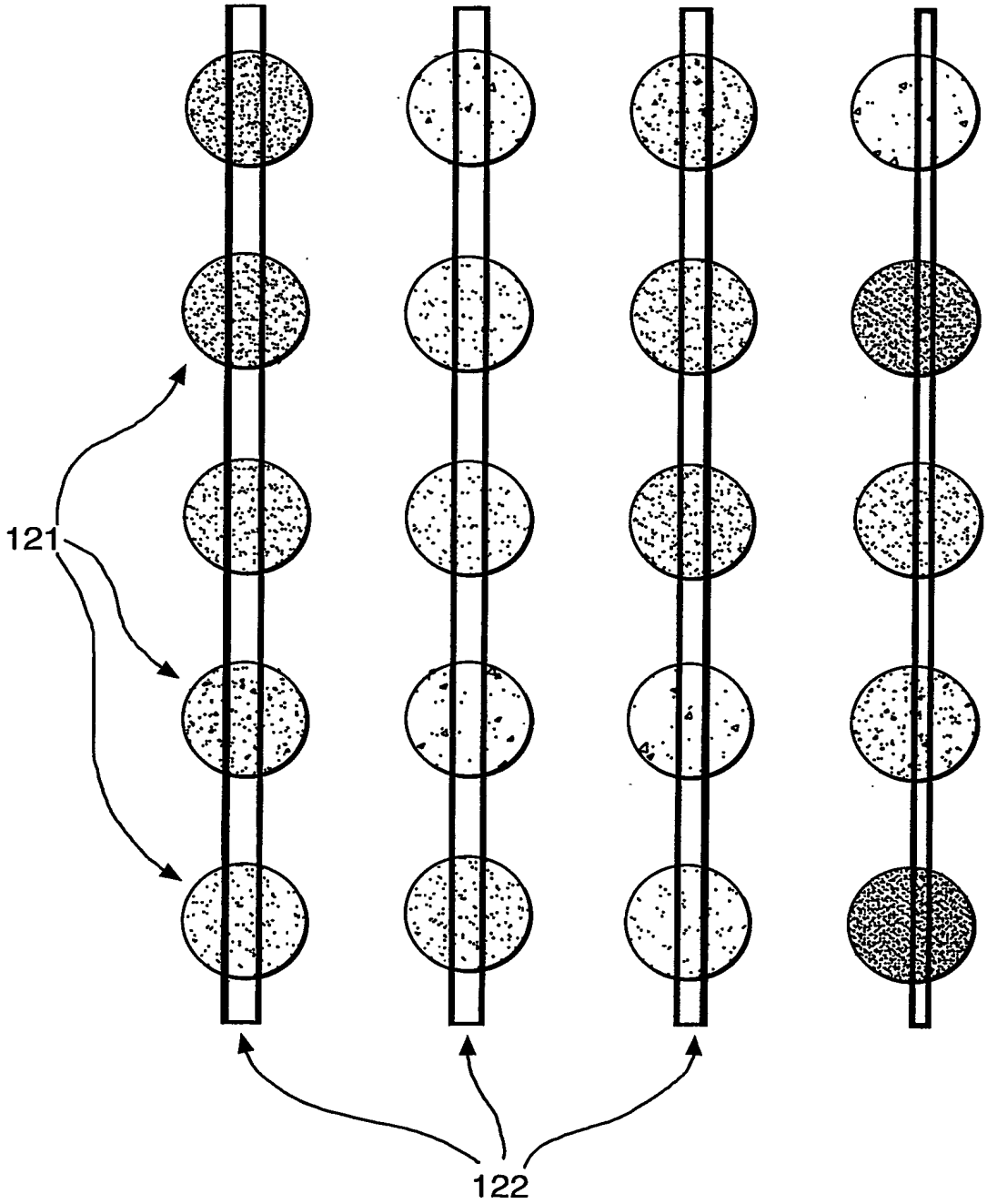

Baseline in physiological buffer
EDC/NHS activation
Change to acidic buffer
Streptavidin binding
Change to physiological buffer
Ethanolamine
Acidic wash
Baseline in physiological buffer
Time
Δn*10-6
12000
10000
8000
6000
4000
2000
0
-2000
-4000
-6000

Fig.12.



INTERNATIONAL SEARCH REPORT

Internati[illegible]pplication No
PCT/GB 03/03803

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/55 G01N21/77

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2003	02/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Consalvo, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
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